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(57) Abstract

It has been discovered that the incorporation of gases, especially fluorinated gases such as perfluorocarbons, into microparticles formed from the combination of a natural or synthetic polymer and lipid have significantly enhanced echogenicity as compared with microparticles not including the lipid. Compounds other than lipids which are hydrophobic and limit the penetration and/or uptake of water into the microparticles can also be incorporated into the microparticles to enhance echogenicity. In the preferred embodiment, the polymers are synthetic biodegradable polymers. The microparticles are manufactured with a diameter suitable for the targeted tissue to be imaged, for example, with a diameter of between 0.5 and 8 microns for intravascular administration, and a diameter of between 0.5 and 5 mm for oral administration for imaging of the gastrointestinal tract or other lumens. Preferred polymers are polyhydroxy acids such as polylactic acid-co-glycolic acid, most preferably conjugated to polyethylene glycol or other materials inhibiting uptake by the reticuloendothelial sys-tem (RES). The most preferred lipids are phospholipids, preferably dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), diarachidoylphosphatidylcholine (DAPC), dibehenoylphosphatidylcholine (DBPC), ditricosanoylphosphatidylcholine, dilignoceroyl-phatidylcholine (DLPC), incorporated at a ratio of between 0.01-30 (w lipid/w polymer), most preferably between 0.1-10 (w lipid/w polymer). Microparticles for imaging using other detectable agents can be similarly manufactured.

POLYMER-LIPID MICROENCAPSULATED GASES
FOR USE AS IMAGING AGENTS

Background of the Invention

The present invention is generally in the area of diagnostic

5   imaging agents, and is particularly directed to microparticulate ultrasound

imaging contrast agents having lipid incorporated therein to maintain the

echogenicity over time.

When using ultrasound to obtain an image of the internal organs

and structures of a human or animal, ultrasound waves, waves of sound

10   energy at a frequency above that discernable by the human ear, are

reflected as they pass through the body. Different types of body tissue

reflect the ultrasound waves differently and the reflections that are

produced by the ultrasound waves reflecting off different internal

structures are detected and converted electronically into a visual display.

15   For some medical conditions, obtaining a useful image of the

organ or structure of interest is especially difficult because the details of

the structure are not adequately discernible from the surrounding tissue in

an ultrasound image produced by the reflection of ultrasound waves

absent a contrast-enhancing agent. Detection and observation of certain

20   physiological and pathological conditions may be substantially improved

by enhancing the contrast in an ultrasound image by infusing an agent into

an organ or other structure or interest. In other cases, detection of the

movement of the contrast-enhancing agent itself is particularly important.

For example, a distinct blood flow pattern that is known to result from

25   particular cardiovascular abnormalities may only be discernible by

infusing a contrasting agent into the bloodstream and observing the

dynamics of the blood flow.

Materials that are useful as ultrasound contrast agents operate by

having an effect on ultrasound waves as they pass through the body and

30   are reflected to create the image from which a medical diagnosis is made.

Different types of substances affect ultrasound waves in different ways

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and to varying degrees. Moreover, certain of the effects caused by contrast-enhancing agents are more readily measured and observed than others. In selecting an ideal composition for a contrast-enhancing agent, one would prefer the substance that has the most dramatic effect on the

5   ultrasound wave as it passed through the body. Also, the effect on the ultrasound wave should be easily measured. There are three main contrast-enhancing effects which can be seen in an ultrasound image: backscatter, beam attenuation, and speed of sound differential.

BACKSCATTER: When an ultrasound wave that is passing

10   through the body encounters a structure, such as an organ or other body tissue, the structure reflects a portion of the ultrasound wave. Different structures within the body reflect ultrasound energy in different ways and in varying strengths. This reflected energy is detected and used to generate an image of the structures through which the ultrasound wave

15   has passed. The term "backscatter" refers to the phenomena in which ultrasound energy is scattered back towards the source by a substance with certain physical properties.

It has long been recognized that the contrast observed in an ultrasound image may be enhanced by the presence of substances known

20   to cause a large amount of backscatter. When such a substance is administered to a distinct part of the body, the contrast between the ultrasound image of this part of the body and the surrounding tissues not containing the substance is enhanced. It is well understood that, due to their physical properties, different substances cause backscatter in varying

25   degrees. Accordingly, the search for contrast-enhancing agents has focused on substances that are stable and non-toxic and that exhibit the maximum amount of backscatter.

The capability of a substance to cause backscatter of ultrasound energy depends on characteristics of the substance such as its ability to be

30   compressed. When examining different substances, it is useful to compare one particular measure of the ability of a substance to cause backscatter known as the "scattering cross-section." The scattering cross-

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section of a particular substance is proportional to the radius of the
scatterer, and also depends on the wavelength of the ultrasound energy
and on other physical properties of the substance, J. Ophir and K.J.
Parker, *Contrast Agents in Diagnostic Ultrasound*, Ultrasound in
5　　Medicine & Biology, vol. IS, n. 4, p. 319, 323 (1989).

　　　　In evaluating the utility of different substances as image
contrasting agents, one can calculate which agents should have the higher
scattering cross-section and, accordingly, which agents should provide the
greatest contrast in an ultrasound image. It can be assumed that the
10　　compressibility of a solid particle is much less than that of the
surrounding medium and that the density of the particle is much greater.
Using this assumption. the scattering cross section of a solid particle
contrast-enhancing agent has been estimated as 1.75 (Ophir and Parker,
supra, at 325). For a pure liquid scatterer. the adiabatic compressibility
15　　and density of the scatterer and the surrounding medium are likely to be
approximately equal. which would yield the result that liquids would have
a scattering cross-section of zero. However, liquids may exhibit some
backscatter if large volumes of a liquid agent are present. For example,
if a liquid agent passes from a very small vessel to a very large one such
20　　that the liquid occupies substantially all of the vessel. the liquid may
exhibit measurable backscatter. Nevertheless, it is appreciated by those
skilled in the art that pure liquids are relatively inefficient scatterers
compared to free gas microbubbles.

　　　　BEAM ATTENUATION: Another effect which can be observed
25　　from the presence of certain solid contrast-enhancing agents, is the
attenuation of the ultrasound wave. Image contrast has been observed in
conventional imaging due to localized attenuation differences between
certain tissue types. K.J. Parker and R.C. Wang, "Measurement of
Ultrasonic Attenuation Within Regions selected from B-Scan Images,"
30　　*IEEE Trans. Biomed. Enar. BME* 30(8), p. 431-37 (1983); K.J. Parker,
R.C. Wang, and R.M. Lerner, "Attenuation of Ultrasound Magnitude and
Frequency Dependence for Tissue Characterization," Radiology, 153(3),

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p. 785-88 (1984). It has been hypothesized that measurements of the attenuation of a region of tissue taken before and after infusion of an agent may yield an enhanced image. However, techniques based on attenuation contrast as a means to measure the contrast enhancement of a

5   liquid agent are not well-developed and, even if fully developed, may suffer from limitations as to the internal organs or structures with which this technique can be used. For example, it is unlikely that a loss of attenuation due to liquid contrast agents could be observed in the image of the cardiovascular system because of the high volume of liquid contrast

10   agent that would need to be present in a given vessel before a substantial difference in attenuation could be measured.

Absorption of energy by the particles occurs by a mechanism referred to as "relative motion." The change in attenuation caused by relative motion can be shown to increase linearly with particle

15   concentration and as the square of the density difference between the particles and the surrounding medium. K.J. Parker, et al., "A Particulate Contrast Agent with Potential for Ultrasound Imaging of Liver," *Ultrasound in Medicine & Biology*, Vol. 13, No. 9, p. 555, 561 (1987). Therefore, where substantial accumulation of solid particles occurs,

20   attenuation contrast may be a viable mechanism for observing image contrast enhancement although the effect is of much smaller magnitude than the backscatter phenomenon and would appear to be of little use in cardiovascular diagnoses.

SPEED OF SOUND DIFFERENTIAL: An additional technique

25   to enhance contrast in an ultrasound image has been proposed based on the fact that the speed of sound varies depending on the media through which it travels. Therefore, if a large enough volume of an agent, through which the speed of sound is different than the surrounding tissue, can be infused into a target area, the difference in the speed of sound

30   through the target area may be measurable.

In summary, diagnostic ultrasound is a powerful, non-invasive tool that can be used to obtain information on the internal organs of the body.

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The advent of grey scale imaging and color Doppler have greatly advanced the scope and resolution of the technique. Although techniques for carrying out diagnostic ultrasound have improved significantly, and for making and using contrast agents, there is still a need to enhance the
5   resolution of the imaging for cardiac perfusion and cardiac chambers, solid organs, renal perfusion; solid organ perfusion; and Doppler signals of blood velocity and flow direction during real-time imaging.

A variety of natural and synthetic polymers have been used to encapsulate imaging contrast agents, such as air. Schneider et al., Invest.
10   Radiol., Vol. 27, pp. 134-139 (1992) describes three micron, air-filled polymeric particles. These particles were reported to be stable in plasma and under applied pressure. However, at 2.5 MHz, their echogenicity was low. Another type of microbubble suspension has been obtained from sonicated albumin. Feinstein et al., J. Am. Coll. Cardiol., Vol. 11,
15   pp. 59-65 (1988). Feinstein describes the preparation of microbubbles that are appropriately sized for transpulmonary passage with excellent stability in vitro. However, these microbubbles are short-lived in vivo, having a half life on the order of a few seconds (which is approximately equal to one circulation pass) because of their instability under pressure.
20   Gottlieb, S. et al., J. Am. Soc. Echo., Vol. 3, pp. 328 (1990), Abstract; and Shapiro, J.R. et al., J. Am. Coll. Cardiol., Vol. 16, pp. 1603-1607 (1990). Gelatin-encapsulated air bubbles have been described by Carroll et al. (Carroll, B.A. et al., Invest. Radiol., Vol. 15, pp. 260-266 (1980), and Carroll, B.A. et al., Radiology, Vol. 143, pp. 747-750 (1982)), but
25   due to their large sizes (12 and 80 μm) they would not be likely to pass through pulmonary capillaries. Gelatin-encapsulated microbubbles have also been described in PCT/US80/00502 by Rasor Associates, Inc. These are formed by "coalescing" the gelatin.

Microbubbles stabilized by microcrystals of galactose (SHU 454
30   and SHU 508) have also been reported by Fritzch et al. Fritzsch, T. et al., Invest. Radiol. Vol. 23 (Suppl 1), pp. 302-305 (1988); and Fritzsch, T. et al., Invest. Radiol., Vol. 25 (Suppl 1), 160-161 (1990). The

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microbubbles last up to 15 minutes *in vitro* but less than 20 seconds *in vivo*. Rovai, D. *et al.*, J. Am. Coll. Cardiol., Vol. 10, pp. 125-134 (1987); and Smith, M. *et al.*, J. Am. Coll. Cardiol., Vol. 13, pp. 1622-1628 (1989). Gas microbubbles encapsulated within a shell of a

5   fluorine-containing material is described in WO 96/04018 by Molecular Biosystems, Inc.

European Patent Application No. 90901933.5 by Schering Aktiengesellschaft discloses the preparation and use of microencapsulated gas or volatile liquids for ultrasound imaging, where the microcapsules

10   are formed of synthetic polymers or polysaccharides. European Patent Application No. 91810366.4 by Sintetica S.A. (0 458 745 A1) discloses air or gas microballoons bounded by an interfacially deposited polymer membrane that can be dispersed in an aqueous carrier for injection into a host animal or for oral, rectal, or urethral administration, for therapeutic

15   or diagnostic purposes. WO 92/18164 by Delta Biotechnology Limited describes the preparation of microparticles by spray drying under very controlled conditions as to temperature, rate of spraying, particle size, and drying conditions, of an aqueous protein solution to form hollow spheres having gas entrapped therein, for use in imaging. WO 93/25242

20   describes the synthesis of microparticles for ultrasonic imaging consisting of a gas contained within a shell of polycyanoacrylate or polyester. WO 92/21382 discloses the fabrication of microparticle contrast agents which include a covalently bonded matrix containing a gas, wherein the matrix is a carbohydrate. U.S. Patent Nos. 5,334,381, 5,123,414 and 5,352,435 to

25   Unger describe liposomes for use as ultrasound contrast agents, which include gases, gas precursors, such as a pH activated or photo-activated gaseous precursor, as well as other liquid or solid contrast enhancing agents.

U.S. Patent No. 5,393,524 to Quay discloses the use of agents,

30   including fluorocarbons, for enhancing the contrast in an ultrasound image. The agents consist of extremely small bubbles, or microbubbles, of selected gases, which exhibit long life spans in solution and are small

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enough to traverse the lungs, enabling their use in ultrasound imaging of
the cardiovascular system and other vital organs.   WO 95/23615 by
Nycomed discloses microcapsules for imaging which are formed by
coacervation of a solution, for example, a protein solution, containing a

5   perfluorocarbon. PCT/US94/08416 by Massachusetts Institute of
Technology discloses microparticles formed of polyethylene glycol-
poly(lactide-co-glycolide) block polymers having imaging agents
encapsulated therein, including gases such as air and perfluorocarbons.
As described in WO 94/16739 by Sonus Pharmaceuticals, Inc., while

10   solids and liquids reflect sound to a similar degree. gases are known to be
more efficient and are the preferred media for use as ultrasound contrast
agents. In fact, as shown by example 12 of the Sonus PCT application,
protein microcapsules were dismissed as raising safety concerns (as well
as efficacy issues) when administered to mini-pigs, as compared to

15   emulsions or colloidal suspensions.

None of these describe microparticles which can be detected using
other methods for detection, such as x-ray, positron or photon emission
tomography, or magnetic resonance imaging.

In all of these cases it is desirable to enhance the echogenicity of

20   the imaging agent. in conjunction with enhancing or maintaining the
stability and ease of manufacturing of the imaging agent. One way in
which to increase the echogenicity of a microparticle is to increase the
time encapsulated gases remain in the circulating microparticles.
Unfortunately, in most cases, the gases diffuse out rapidly, regardless of

25   the nature of the gas or the encapsulating material, particularly in the
aqueous environment of the vascular circulation.

It is therefore an object of the present invention to provide
microparticles with significantly enhanced echogenicity. It is another
object of the invention to provide microparticles containing an imaging

30   agent that can persist for more than a few circulation times *in vivo*. It is
a further object of the present invention to provide microparticles which

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retain encapsulated gas for longer periods of time, therefore increasing the *in vivo* echogenicity of the microparticles.

It is another object of the invention to provide microparticles which contain an imaging agent. It is a further object of the invention to
5   provide microparticles containing imaging agents that are targeted to specific regions of the body. It is still another object of the present invention to provide methods for making microparticles having imaging agents entrapped therein.

Summary of the Invention

10   It has been discovered that the incorporation of gases, especially fluorinated gases such as perfluorocarbons, into microparticles formed from the combination of a natural or synthetic polymer and lipid have significantly enhanced echogenicity as compared with microparticles not including the lipid. Compounds other than lipids which are hydrophobic
15   and limit the diffusion of water into the microparticles can also be incorporated into the microparticles to enhance echogenicity. In the preferred embodiment, the polymers are synthetic biodegradable polymers. The microparticles are manufactured with a diameter suitable for the targeted tissue to be imaged, for example, with a diameter of
20   between 0.5 and 8 microns for intravascular administration, and a diameter of between 0.5 and 5 mm for oral administration for imaging of the gastrointestinal tract or other lumens. Preferred polymers are polyhydroxy acids such as polylactic acid-co-glycolic acid, polylactide or polyglycolide, most preferably conjugated to polyethylene glycol or other
25   materials inhibiting uptake by the reticuloendothelial system (RES). The most preferred lipids are phospholipids, preferably dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), diarachidoylphosphatidylcholine (DAPC), dibehenoylphosphatidylcholine (DBPC), ditricosanoylphosphatidylcholine
30   (DTPC), dilignoceroylphatidylcholine (DLPC), incorporated at a ratio of

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between 0.01-30 (w lipid/w polymer), most preferably between 0.1-10 (w lipid/w polymer).

Adhesion of these microparticles can be enhanced or reduced through the selection of bioadhesive polymers. For example, adhesion
5   can be enhanced in the case where the polymer is used for oral administration. Targeting can also be achieved by selection of the polymer or incorporation within or coupling to the polymer of ligands which specifically bind to particular tissue types or cell surface molecules. Additionally, ligands may be attached to the microspheres which effect
10   the charge, lipophilicity or hydrophilicity of the particle. The polymeric microparticles are useful in a variety of diagnostic imaging procedures including ultrasound imaging, magnetic resonance imaging, fluoroscopy, x-ray, and computerized tomography. The microspheres may be used in a variety of imaging applications including cardiology applications, blood
15   perfusion applications as well as for organ and peripheral vein imaging.

Brief Description of the Drawing

Figure 1 is a graph of the effect of carbon chain length of lipid incorporated into polymeric microparticles, plotted as degree of backscattering over time (minutes) for lecithin (solid circles), DPPC (open
20   squares), DSPC (open diamonds), and DAPC (X).

Detailed Description of the Invention

Methods are provided for the synthesis of polymeric delivery systems consisting of polymer-lipid microparticles which contain gases, especially perfluorocarbons. The microparticles are useful in a variety of
25   diagnostic ultrasound imaging applications, particularly in ultrasound procedures such as blood vessel imaging and echocardiography. The incorporation of additional lipid significantly increases the echogenicity as compared with the same polymeric microparticles without the additional lipid.

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Processes and Reagents for Making Microparticles

As used herein, the term microparticle includes microspheres and microcapsules, as well as microparticles, unless otherwise specified. Microparticles may or may not be spherical in shape. Microcapsules are

5   defined as microparticles having an outer polymer shell surrounding a core of another material, in this case, a gas. Microspheres are generally solid polymeric spheres, which can include a honeycombed structure formed by pores through the polymer which are filled with a gas for imaging purposes, as described below.

10   **Polymers**

Both non-biodegradable and biodegradable matrices can be used mixed with lipids for delivery of gases, although biodegradable matrices are preferred, particularly for intravenous injection. Non-erodible polymers may be used for oral administration. Synthetic polymers are

15   preferred due to more reproducible synthesis and degradation. The polymer is selected based on the time required for *in vivo* stability, i.e., that time required for distribution to the site where imaging is desired, and the time required for imaging. In one embodiment, microparticles with an *in vivo* stability of between about 20 to 30 minutes or more may

20   be fabricated, for example for use in applications such as echocardiography, neurosonography, hysterosalpingography, and diagnostic procedures on solid organs. The *in vivo* stability of the contrast agent-encapsulated microparticles can be adjusted during the production by using polymers such as polylactide co glycolide

25   copolymerized with polyethylene glycol (PEG). PEG if exposed on the external surface may elongate the time these materials circulate since it is hydrophilic.

Representative synthetic polymers are: poly(hydroxy acids) such as poly(lactic acid), poly(glycolic acid), and poly(lactic acid-co-glycolic

30   acid), polyglycolides, polylactides, polylactide co-glycolide copolymers and blends, polyanhydrides, polyorthoesters, polyamides, polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene

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glycols such as poly(ethylene glycol), polyalkylene oxides such as

poly(ethylene oxide), polyalkylene terepthalates such as poly(ethylene

terephthalate), polyvinyl alcohols, polyvinyl ethers, polyvinyl esters,

polyvinyl halides such as poly(vinyl chloride), polyvinylpyrrolidone,

5   polysiloxanes, poly(vinyl alcohols), poly(vinyl acetate), polystyrene,

polyurethanes and co-polymers thereof, derivativized celluloses such as

alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters,

nitro celluloses, methyl cellulose, ethyl cellulose, hydroxypropyl

cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl

10   cellulose, cellulose acetate, cellulose propionate, cellulose acetate

butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose

triacetate, and cellulose sulphate sodium salt (jointly referred to herein as

"synthetic celluloses"), polymers of acrylic acid, methacrylic acid or

copolymers or derivatives thereof including esters, poly(methyl

15   methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate),

poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl

methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate),

poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate),

and poly(octadecyl acrylate) (jointly referred to herein as "polyacrylic

20   acids"), poly(butyric acid), poly(valeric acid), and poly(lactide-co-

caprolactone), copolymers and blends thereof. As used herein,

"derivatives" include polymers having substitutions, additions of chemical

groups, for example, alkyl, alkylene, hydroxylations, oxidations, and

other modifications routinely made by those skilled in the art.

25   Examples of preferred non-biodegradable polymers include

ethylene vinyl acetate, poly(meth)acrylic acid, polyamides, copolymers

and mixtures thereof.

            Examples of preferred biodegradable polymers include polymers of

hydroxy acids such as lactic acid and glycolic acid, polylactide,

30   polyglycolide, polylactide-co-glycolide, and copolymers with PEG,

polyanhydrides, poly(ortho)esters, polyurethanes, poly(butyric acid),

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poly(valeric acid), poly(lactide-co-caprolactone), blends and copolymers thereof.

Examples of preferred natural polymers include proteins such as albumin and prolamines, for example, zein, and polysaccharides such as alginate, cellulose and polyhydroxyalkanoates, for example, polyhydroxybutyrate.

Bioadhesive polymers of particular interest for use in imaging of mucosal surfaces, as in the gastrointestinal tract, include polyanhydrides, polyacrylic acid, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

Solvents

As defined herein, the polymer solvent is an organic solvent that is volatile or has a relatively low boiling point or can be removed under vacuum and which is acceptable for administration to humans in trace amounts, such as methylene chloride. Other solvents, such as ethyl acetate, ethanol, methanol, dimethyl formamide (DMF), acetone, acetonitrile, tetrahydrofuran (THF), acetic acid, dimethyle sulfoxide (DMSO) and chloroform also may be utilized, or combinations thereof. In general, the polymer is dissolved in the solvent to form a polymer solution having a concentration of between 0.1 and 60% weight to volume (w/v), more preferably between 0.25 and 30%.

Hydrophobic Compounds

Lipids

In general, incorporation of compounds which are hydrophobic and, in an effective amount, thereby limit penetration and/or uptake of water by the microparticles are effective in increasing echogenicity of polymeric microparticles having gas encapsulated therein, especially fluorinated gases such as perfluorocarbons. Lipids which may be used to

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stabilize gas inside the polymeric microparticles include but are not
limited to the following classes of lipids: fatty acids and derivatives,
mono-, di and triglycerides, phospholipids, sphingolipids, cholesterol and
steroid derivatives, terpenes and vitamins. Fatty acids and derivatives

5   thereof may include but are not limited to saturated and unsaturated fatty
acids, odd and even number fatty acids, cis and trans isomers, and fatty
acid derivatives including alcohols, esters, anhydrides, hydroxy fatty acids
and prostaglandins. Saturated and unsaturated fatty acids that may be
used include, but are not limited to, molecules that have between 12

10   carbon atoms and 22 carbon atoms in either linear or branched form.
Examples of saturated fatty acids that may be used include, but are not
limited to, lauric, myristic, palmitic, and stearic acids. Examples of
unsaturated fatty acids that may be used include, but are not limited to,
lauric, physeteric, myristoleic, palmitoleic, petroselinic, and oleic acids.

15   Examples of branched fatty acids that may be used include, but are not
limited to, isolauric, isomyristic, isopalmitic, and isostearic acids and
isoprenoids. Fatty acid derivatives include 12-(((7'-
diethylaminocoumarin-3 yl)carbonyl)methylamino)-octadecanoic acid; N-
[12-(((7'diethylaminocoumarin-3-yl) carbonyl)methyl-amino)

20   octadecanoyl]-2-aminopalmitic acid, N succinyl-
dioleoylphosphatidylethanol amine and palmitoyl-homocysteine; and/or
combinations thereof. Mono, di and triglycerides or derivatives thereof
that may be used include, but are not limited to molecules that have fatty
acids or mixtures of fatty acids between 6 and 24 carbon atoms,

25   digalactosyldiglyceride, 1,2-dioleoyl-sn-glycerol;1,2-cdipalmitoyl-sn-3
succinylglycerol; and 1,3-dipalmitoyl-2-succinylglycerol.

Phospholipids which may be used include but are not limited to
phosphatidic acids, phosphatidyl cholines with both saturated and
unsaturated lipids, phosphatidyl ethanolamines, phosphatidylglycerols,

30   phosphatidylserines, phosphatidylinositols, lysophosphatidyl derivatives,
cardiolipin, and β-acyl-y-alkyl phospholipids. Examples of phospholipids
include, but are not limited to, phosphatidylcholines such as

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dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine,
dipentadecanoylphosphatidylcholine dilauroylphosphatidylcholine,
dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine
(DSPC), diarachidoylphosphatidylcholine (DAPC),

5   dibehenoylphosphatidylcholine (DBPC), ditricosanoylphosphatidylcholine
(DTPC), dilignoceroylphatidylcholine (DLPC); and
phosphatidylethanolamines such as dioleoylphosphatidylethanolamine or 1-
hexadecyl-2--palmitoylglycerophosphoethanolamine. Synthetic
phospholipids with asymmetric acyl chains (e.g., with one acyl chain of 6

10   carbons and another acyl chain of 12 carbons) may also be used.

Sphingolipids which may be used include ceramides,
sphingomyelins, cerebrosides, gangliosides, sulfatides and lysosulfatides.
Examples of Sphinglolipids include, but are not limited to, the
gangliosides GM1 and GM2.

15   Steroids which may be used include but are not limited to
cholesterol, cholesterol sulfate, cholesterol hemisuccinate, 6-(5-cholesterol
3β-yloxy) hexyl-6-amino-6-deoxy-l-thio-α-D-galactopyranoside, 6-(5-
cholesten-3 β-tloxy)hexyl-6-amino-6-deoxyl-1-thio-α-D mannopyranoside
and cholesteryl)4'-trimethyl 35 ammonio)butanoate.

20   Additional lipid compounds which may be used include tocopherol
and derivatives, and oils and derivatized oils such as stearlyamine.

A variety of cationic lipids such as DOTMA, N-[1-(2,3-
dioleoyloxy)propyl-N,N,N-trimethylammonium chloride; DOTAP, 1,2-
dioleoyloxy-3-(trimethylammonio) propane; and DOTB, 1,2-dioleoyl-3-

25   (4'-trimethyl-ammonio) butanoyl-sn glycerol may be used.

The most preferred lipids are phospholipids, preferably DPPC,
DDSPC, DAPC, DSPC, DTPC, DBPC, DLPC and most preferably
DPPC, DAPC and DBPC.

The lipid content ranges from .01-30 (w lipid/w polymer); most

30   preferably between 0.1-10 (w lipid/w polymer).

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Other Hydrophobic Compounds

Other preferred hydrophobic compounds include amino acids such as tryptophane, tyrosine, isoleucine, leucine, and valine, aromatic compounds such as an alkyl paraben, for example, methyl paraben, and
5   benzoic acid.

Imaging Agents

Gases

Any biocompatible or pharmacologically acceptable gas can be incorporated into the microparticles. The term gas refers to any
10   compound which is a gas or capable of forming a gas at the temperature at which imaging is being performed. The gas may be composed of a single compound such as oxygen, nitrogen, xenon, argon, nitrogen or a mixture of compounds such as air. Fluorinated gases are preferred. Examples of fluorinated gases include CF_4, C_2F_6, C_3F_8, C_4F_8, SF_6, C_2F_4,
15   and C_3F_6. Perfluoropropane is particularly preferred because it provides an insoluble gas that will not condense at the temperature of use and is pharmacologically acceptable.

Other Imaging Agents

Other imaging agents can be incorporated in place of a gas, or in
20   combination with the gas. Imaging agents which may be utilized include commercially available agents used in positron emission tomography (PET), computer assisted tomography (CAT), single photon emission computerized tomography, x-ray, fluoroscopy, and magnetic resonance imaging (MRI). Microparticles loaded with these agents can be detected
25   using standard techniques available in the art and commercially available equipment.

Examples of suitable materials for use as contrast agents in MRI include the gatalinium chelates currently available, such as diethylene triamine pentacetic acid (DTPA) and gatopentotate dimeglumine, as well
30   as iron, magnesium, manganese, copper and chromium.

Examples of materials useful for CAT and x-rays include iodine based materials for intravenous administration, such as ionic monomers

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typified by diatrizoate and iothalamate, non-ionic monomers such as iopamidol, isohexol, and ioversol, non-ionic dimers, such as iotrol and iodixanol, and ionic dimers, for example, ioxagalte. Other useful materials include barium for oral use.

Microparticles and Methods for Manufacture Thereof

In the most preferred embodiment, the microparticles are produced by spray drying. Other techniques can be used, such as solvent extraction, hot melt encapsulation, and solvent evaporation, as discussed below. A major criteria is that the polymer must be dissolved or melted with the lipid, prior to forming the microparticle. Although described specifically with reference to incorporation of a lipid, it is understood that other useful hydrophobic compounds could be substituted for the lipid.

In a preferred embodiment, the gas is then replaced by applying a stream of the desired gas to, or pulling a vacuum on, the microparticles to remove the encapsulated gas, then filling with the desired gas.

a.   Solvent Evaporation. In this method the polymer and lipid are dissolved in a volatile organic solvent such as methylene chloride. A pore forming agent as a solid or in solution may be added to the solution, for use in forming microparticles which are to incorporate gas as the imaging agent. If other imaging agents are to be incorporated, the imaging agent can be added as either a solid or in solution to the polymer solution. The mixture is sonicated or homogenised and the resulting dispersion or emulsion is added to an aqueous solution that contains a surface active agent such as TWEEN™ 20, TWEEN™ 80, PEG or poly(vinyl alcohol) and homogenised to form an emulsion. The resulting emulsion is stirred until most of the organic solvent evaporates, leaving microparticles. Several different polymer concentrations can be used (0.05-0.60 g/ml). Microparticles with different sizes (1-1000 microns) and morphologies can be obtained by this method. This method is useful for relatively stable polymers like polyesters.

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Solvent evaporation is described by E. Mathiowitz, et al., J. Scanning Microscopy, 4, 329 (1990); L.R. Beck, et al., Fertil. Steril., 31, 545 (1979); and S. Benita, et al., J. Pharm. Sci., 73, 1721 (1984).

However, labile polymers, such as polyanhydrides, may degrade
5   during the fabrication process due to the presence of water. For these polymers, the following two methods, which are performed in completely organic solvents, are more useful.

b. Hot Melt Microencapsulation. In this method, the polymer and lipid are first melted and then mixed with the solid particles of the pore
10   forming agent or solid or liquid diagnostic agent. The mixture is suspended in a non-miscible solvent (like silicon oil), and, while stirring continuously, heated to 5°C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microparticles are washed by decantation with a
15   polymer non-solvent such as petroleum ether to give a free-flowing powder. Microparticles with sizes between one to 1000 microns can be obtained with this method. The external surfaces of particles prepared with this technique are usually smooth and dense. This procedure is used to prepare microparticles made of polyesters and polyanhydrides.
20   However, this method is limited to polymers with molecular weights between 1000-50,000.

Hot-melt microencapsulation is described by E. Mathiowitz, et al., Reactive Polymers, 6, 275 (1987). Polyanhydrides, for example, made of bis-carboxyphenoxypropane and sebacic acid with molar ratio of 20:80
25   (P(CPP-SA) 20:80) (Mw 20,000), can be prepared by hot-melt microencapsulation or for example, poly(fumaric-co-sebacic) (20:80) (Mw 15,000) microparticles, can be prepared by hot-melt microencapsulation.

c. Solvent Removal. This technique was primarily designed for polyanhydrides. In this method, the pore forming agent is dispersed or
30   dissolved in a solution of the selected polymer and lipid in a volatile organic solvent like methylene chloride. This mixture is suspended by stirring in an organic oil (such as silicon oil) to form an emulsion.

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Unlike solvent evaporation, this method can be used to make microparticles from polymers with high melting points and different molecular weights. The external morphology of particles produced with this technique is highly dependent on the type of polymer used.

5   d. <u>Spray Drying of Microparticles.</u> Microparticles can be produced by spray drying by dissolving a biocompatible polymer and lipid in an appropriate solvent, dispersing a pore forming agent into the polymer solution, and then spray drying the polymer solution, to form microparticles. As defined herein, the process of "spray drying" a

10   solution of a polymer and a pore forming agent refers to a process wherein the solution is atomized to form a fine mist and dried by direct contact with hot carrier gases. Using spray drying apparatus available in the art, the polymer solution may be delivered through the inlet port of the spray drier, passed through a tube within the drier and then atomized

15   through the outlet port. The temperature may be varied depending on the gas or polymer used. The temperature of the inlet and outlet ports can be controlled to produce the desired products.

          The size of the particulates of polymer solution is a function of the nozzle used to spray the polymer solution, nozzle pressure, the flow rate,

20   the polymer used, the polymer concentration, the type of solvent and the temperature of spraying (both inlet and outlet temperature) and the molecular weight. Generally, the higher the molecular weight, the larger the particle size, assuming the concentration is the same. Typical process parameters for spray drying are as follows: polymer concentration =

25   0.005-0.20 g/ml, inlet temperature = 30-1000°C, outlet temperature = 20-100°C, polymer flow rate = 5-200 ml/min., and nozzle diameter = 0.2-4 mm ID. Microparticles ranging in diameter between one and ten microns can be obtained with a morphology which depends on the selection of polymer, concentration, molecular weight and spray flow.

30   If the imaging agent is a solid, the agent may be encapsulated as solid particles which are added to the polymer solution prior to spraying, or the imaging agent can be dissolved in an aqueous solution which then

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is emulsified with the polymer solution prior to spraying, or the solid may be cosolubilized together with the polymer in an appropriate solvent prior to spraying.

    e. Hydrogel Microparticles. Microparticles made of gel-type polymers, such as polyphosphazene or polymethylmethacrylate, are produced by dissolving the polymer in an aqueous solution, suspending if desired a pore forming agent and suspending a lipid in the mixture, homogenizing the mixture, and extruding the material through a microdroplet forming device, producing microdroplets which fall into a hardening bath consisting of an oppositely charged ion or polyelectrolyte solution, that is slowly stirred. The advantage of these systems is the ability to further modify the surface of the microparticles by coating them with polycationic polymers, like polylysine after fabrication. Microparticle particles are controlled by using various size extruders.

    Additives to Facilitate Microparticulate Formation

    A variety of surfactants may be added during the synthesis of the image agent-containing microparticles. Exemplary emulsifiers or surfactants which may be used (0.1-5% by weight) include most physiologically acceptable emulsifiers. Examples include natural and synthetic forms of bile salts or bile acids, both conjugated with amino acids and unconjugated such as taurodeoxycholate, and cholic acid.

    Pore forming agents can be included in an amount of between 0.01% and 90% weight to volume, to increase pore formation. For example, in spray drying, solvent evaporation, a pore forming agent such as a volatile salt, for example, ammonium bicarbonate, ammonium acetate, ammonium chloride or ammonium benzoate or other lyophilizable salt, is first dissolved in water. The solution containing the pore forming agent is then emulsified with the polymer solution to create droplets of the pore forming agent in the polymer. This emulsion is then spray dried or taken through a solvent evaporation/extraction process. After the polymer is precipitated, the hardened microparticles are frozen and lyophilized to remove the pore forming agents.

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Microparticle Size

In a preferred embodiment for the preparation of injectable microparticles capable of passing through the pulmonary capillary bed, the microparticles should have a diameter of between approximately one and ten microns. Larger microparticles may clog the pulmonary bed, and smaller microparticles may not provide sufficient echogenicity. Larger microparticles are useful for administration by routes other than injection, for example oral (for evaluation of the gastrointestinal tract), application to other mucosal surfaces (rectal, vaginal, oral, nasal) or by inhalation. The preferred particle size for oral administration is about 0.5 microns and 5 mm. Useful pharmaceutically acceptable carriers include saline containing glycerol and TWEEN™ 20 and isotonic mannitol containing TWEEN™ 20. Particle size analysis can be performed on a Coulter counter by light microscopy, scanning electron microscopy, or transmittance electron microscopy.

Targeting

The microparticles can be targeted specifically or non-specifically through the selection of the polymer forming the microparticle, the size of the microparticle, and/or incorporation or attachment of a ligand to the microparticles. For example, biologically active molecules, or molecules affecting the charge, lipophilicity or hydrophilicity of the particle, may be attached to the surface of the microparticle. Additionally, molecules may be attached to the microparticles which minimize tissue adhesion, or which facilitate specific targeting of the microparticles *in vivo*. Representative targeting molecules include antibodies, lectins, and other molecules which are specifically bound by receptors on the surfaces of cells of a particular type.

Inhibition of Uptake by the RES

Uptake and removal of the microparticles can also be minimized through the selection of the polymer and/or incorporation or coupling of molecules which minimize adhesion or uptake. For example, tissue adhesion by the microparticle can be minimized by covalently binding

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poly(alkylene glycol) moieties to the surface of the microparticle. The
surface poly(alkylene glycol) moieties have a high affinity for water that
reduces protein adsorption onto the surface of the particle. The
recognition and uptake of the microparticle by the reticulo-endothelial

5   system (RES) is therefore reduced.

       For example, the terminal hydroxyl group of the poly(alkylene
glycol) can be used to covalently attach biologically active molecules, or
molecules affecting the charge, lipophilicity or hydrophilicity of the
particle, onto the surface of the microparticle. Methods available in the

10   art can be used to attach any of a wide range of ligands to the
microparticles to enhance the delivery properties, the stability or other
properties of the microparticles *in vivo*.

       Diagnostic Applications

       Microparticles are typically combined with a pharmaceutically

15   acceptable carrier such as phosphate buffered saline or saline or mannitol,
then an effective amount for detection administered to a patient using an
appropriate route, typically by injection into a blood vessel (i.v.) or
orally. Microparticles containing an encapsulated imaging agent may be
used in vascular imaging, as well as in applications to detect liver and

20   renal diseases, in cardiology applications, in detecting and characterizing
tumor masses and tissues, and in measuring peripheral blood velocity.
The microparticles also can be linked with ligands that minimize tissue
adhesion or that target the microparticles to specific regions of the body
in vivo as described above.

25   The methods and compositions described above will be further
understood with reference to the following non-limiting examples.

       Example 1:   Preparation of Octafluoropropane PEG-PLGA/PLGA
                microparticles with Lecithin.

       3.2 grams of PEG-PLGA (75:25) (IV=0.75 dL/g Birmingham

30   Polymers), 6.4 g PLGA (50:50) (IV= .4 dL/g Henley Chemicals), 23 mg
Lecithin (Spectrum Chemicals), and 193 mg of Palmitic Acid (Spectrum
Chemicals) were dissolved in 190 ml of methylene chloride. 10.8 ml of

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0.70 g/ml ammonium acetate was added to the polymer solution and the
polymer/ ammonium acetate was added to the polymer solution and the
polymer/ammonium acetate mixture was homogenised at 10,000 RPM for
1 minute using a Virtis homogeniser. The solution was pumped at a

5   flowrate of 20 ml/min and sprayed dried using a Bucchi Lab spray dryer.
The inlet temperature was 40 o C. The microparticle powder was
collected and lyophilized on an FTS tray lyophilizer for 120 hours. The
microparticles were aliquoted into 5 ml Purform vials and sealed with
butyl stoppers and crimped. The vials were filled with octofluoropropane

10   at a pressure of 10 psig and purged continuously under the gas for three
minutes. After this point the vials were stored at 4 o C until used. The
particle diameters ranged from 1-10 microns when sized on a coulter
counter with a number average mean of 2.0 microns. Scanning electron
microscopy demonstrated the particles to be generally spherical with

15   smooth surfaces and occasional surface crenulations.

**Example 2:   Preparation of Octafluoropropane PEG-PLGA/PLGA
microparticles with dipalmitoylphosphatidylcholine
(DPPC).**

Microparticles were prepared as in Example 1 except 29.6 mg of

20   dipalmitoylphosphatidylcholine (Avanti, Birmingham Al) was used in
place of the lecithin.

**Example 3:   Preparation of Octafluoropropane PEG-PLGA/PLGA
microparticles with distearoylphosphatidylcholine
(DSPC).**

25   Microparticles were prepared as in Example 2 except that 29.9 mg
of distearoylphosphatidylcholine (Avanti, Birmingham Al) was used in
place of the lecithin.

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Example 4: Preparation of Octafluoropropane PEG-PLGA/PLGA microparticles with diarachidoylphosphatidylcholine (DAPC).

Microparticles were prepared as in Example 2 except that 29.9 mg
5   of diarachidoylphosphatidylcholine (Avanti, Birmingham Al) in place of
the lecithin.

Example 5:   *In Vitro* **Measurement of Microparticle Backscatter.**

The backscatter of the different octafluoropropane containing
polymeric microparticles produced in Examples 1-4 was obtained by
10   exposing 10 microliters of suspension of the microparticles to a focused
ultrasound beam. The backscattered acoustic power as a function of
depth into the sample was determined as follows. A pulser-receiver
(Panametrics® Model 5800) was used to shock excite a focused ultrasound
transducer (2.25 MHz), which sends a pulse of ultrasound into the
15   suspension of microparticles in physiological saline.

The suspension was contained in a cylindrical sample chamber (55
ml of saline) which is situated in a temperature-controlled water bath
adjusted to 37°C. The chamber was positioned 1.5 inches from the
transducer, such that the transducer was focused on the acoustic window
20   of the chamber. Chambers were rotated at 15 rpm to maintain the
microparticles in suspension. The dissolved gas content of the saline was
maintained at approximately 90% of air saturation, as determined by a
dissolved oxygen meter (Orion® model 840). The operation of the
acoustic test system is controlled by a PC running a proprietary program
25   written under LabVIEW® (National Instruments®). The computer
triggered the pulser-receiver to shock excite the ultrasound transducer.

The backscattered signal was received by the same transducer, and
the returned signal was amplified by the pulser-receiver unit. The
amplified signal was passed to a digital oscilloscope (LeCroy® model
30   9310AM) for digitization at 100 MSa/s. The digitized signal was further
processed. The signal was squared, analyzed by FFT and integrated over
the 6 dB bandwidth of the transducer. The acoustic data collected by the

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system was converted to integrated backscattered power (IBP), in arbitrary units, as a function of depth in the microparticulate suspension. The IBP vs depth data from 50 pulses were averaged, the best-fitting straight line through the averaged IBP data determined and the y-intercept

5   which is proportional to the backscatter coefficient was determined. Each sample was tested at 2.5 minute intervals over a total time period of 10 minutes.

The backscatter as a function of time for the four different microparticle lots is shown in Figure 1. Lecithin is a mixture of different

10   chain length phospholipids. As the chain length of the fatty acid attached to the phosphocholine is increased, the magnitude of backscatter is more sustained over a longer period of time indicating increased stability of the octafluoropropane in the microparticles. Using the highly purified phospholipids is also more effective at stabilization of the gas as

15   compared to the mixture of phospholipids contained in lecithin.

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We claim:

1. A method for making microparticles for diagnostic imaging, wherein the microparticles are formed of a biocompatible polymer and have incorporated therein an imaging agent, comprising incorporating into the polymer a hydrophobic compound in an amount decreasing the rate of penetration or uptake of water into the microparticle.

2. The method of claim 1 wherein the hydrophobic compound is incorporated with the polymer at a ratio of between 0.01 and 30 by weight of hydrophobic compound to weight of polymer.

3. The method of claim 2 wherein the hydrophobic compound is a lipid incorporated with the polymer at a ratio of between 0.01 and 30 (weight lipid/weight polymer).

4. The method of claim 1 wherein the lipid is selected from the group consisting of fatty acids and derivatives, mono-, di and triglycerides, phospholipids, sphingolipids, cholesterol and steroid derivatives, oils, vitamins and terpenes.

5. The method of claim 4 wherein the lipid is a phospholipid selected from the group consisting of phosphatidic acids, phosphatidyl cholines with both saturated and unsaturated lipids, phosphatidyl ethanolamines, phosphatidylglycerols, phosphatidylserines, phosphatidylinositols, lysophosphatidyl derivatives, cardiolipin, and β-acyl-y-alkyl phospholipids.

6. The method of claim 5 wherein the phospholipid is selected from the group consisting of dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipentadecanoylphosphatidylcholine dilauroylphosphatidylcholine, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, diarachidoylphosphatidylcholine, dibehenoylphosphatidylcholine, ditricosanoylphosphatidylcholine, dilignoceroylphatidylcholine; and phosphatidylethanolamines.

7. The method of claim 1 wherein the imaging agent is a gas selected from the group consisting of fluorinated gases, oxygen, xenon, argon, helium, and air.

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8. The method of claim 7 wherein the gas is selected from the group consisting of CF_4, C_2F_6, C_3F_8, C_4F_8, SF_6, C_2F_4, and C_3F_6.

9. The method of claim 8 wherein the gas is octafluoropropane.

10. The method of claim 1 wherein the imaging agent is detectable by an imaging technique selected from the group consisting of magnetic resonance imaging, computer tomography, x-ray, positron emission tomography and single photon emission computerized tomography.

11. The method of claim 1 wherein the microparticle is formed of a synthetic polymer.

12. The method of claim 1 wherein the microparticle is formed of a natural polymer.

13. The method of claim 1 wherein the microparticle is formed of a bioadhesive polymer.

14. The method of claim 11 wherein the microparticle is formed of a synthetic polymer selected from the group consisting of poly(hydroxy acids), polyanhydrides, polyorthoesters, polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polysiloxanes, poly(vinyl alcohols), poly(vinyl acetate), polystyrene, polyurethanes and co-polymers thereof, synthetic celluloses, polyacrylic acids, poly(butyric acid), poly(valeric acid), and poly(lactide-co-caprolactone), ethylene vinyl acetate, copolymers and blends thereof.

15. Microparticles for diagnostic imaging, wherein the microparticles are formed of a biocompatible polymer and have incorporated therein an imaging agent, comprising incorporated into the polymer an effective amount of a hydrophobic compound decreasing the rate of penetration or uptake of water into the microparticle.

16. The microparticles of claim 15 wherein the hydrophobic compound is incorporated with the polymer at a ratio of between 0.01 and 30 by weight of hydrophobic compound to weight of polymer.

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17. The microparticles of claim 16 wherein the hydrophobic compound is a lipid incorporated with the polymer at a ratio of between 0.01 and 30 (weight lipid/weight polymer).

18. The microparticles of claim 17 wherein the lipid is selected from the group consisting of fatty acids and derivatives, mono-, di and triglycerides, phospholipids, sphingolipids, cholesterol and steroid derivatives, oils, vitamins and terpenes.

19. The microparticles of claim 18 wherein the lipid is a phospholipid selected from the group consisting of phosphatidic acids, phosphatidyl cholines with both saturated and unsaturated lipids, phosphatidyl ethanolamines, phosphatidylglycerols, phosphatidylserines, phosphatidylinositols, lysophosphatidyl derivatives, cardiolipin, and β-acyl-y-alkyl phospholipids.

20. The microparticles of claim 19 wherein the phospholipid is selected from the group consisting of dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipentadecanoylphosphatidylcholine dilauroylphosphatidylcholine, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, diarachidoylphosphatidylcholine, dibehenoylphosphatidylcholine, ditricosanoylphosphatidylcholine, dilignoceroylphatidylcholine; and phosphatidylethanolamines.

21. The microparticles of claim 15 wherein the imaging agent is a gas selected from the group consisting of fluorinated gases, oxygen, xenon, argon, helium, and air.

22. The microparticles of claim 21 wherein the gas is selected from the group consisting of CF_4, C_2F_6, C_3F_8, C_4F_8, SF_6, C_2F_4, and C_3F_6.

23. The microparticles of claim 22 wherein the gas is octafluoropropane.

24. The microparticles of claim 15 wherein the imaging agent is detectable by an imaging technique selected from the group consisting of magnetic resonance imaging, computer tomography, x-ray, positron emission tomography and single photon emission computerized tomography.

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25. The microparticles of claim 15 wherein the microparticle is formed of a synthetic polymer.

26. The microparticles of claim 25 wherein the polymer is selected from the group consisting of poly(hydroxy acids), polyanhydrides, polyorthoesters, polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polysiloxanes, poly(vinyl alcohols), poly(vinyl acetate), polystyrene, polyurethanes and co-polymers thereof, synthetic celluloses, polyacrylic acids, poly(butyric acid), poly(valeric acid), and poly(lactide-co-caprolactone), ethylene vinyl acetate, copolymers and blends thereof.

27. The microparticles of claim 17 wherein the lipid is liquified with the polymer to form the microparticles.

28. The microparticles of claim 27 wherein the lipid and the polymer are dissolved in a solvent for both, then formed into microparticles.

29. The microparticles of claim 27 wherein the gas is incorporated into the microparticle after the polymer and lipid have solidified.

30. The microparticles of claim 15 wherein the polymer is a natural polymer selected from the group consisting of proteins and polysaccharides.

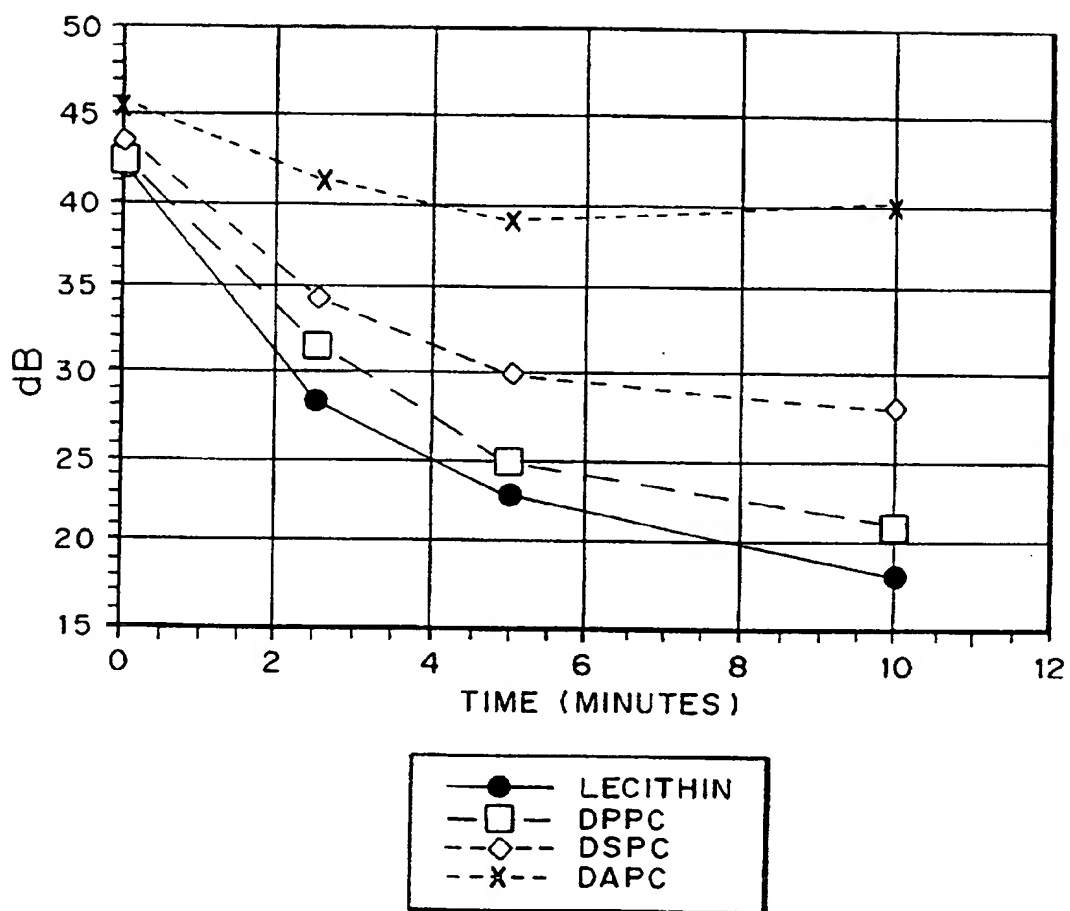FIG. 1